Electronic Supplementary Material (ESI) for Lab on a Chip
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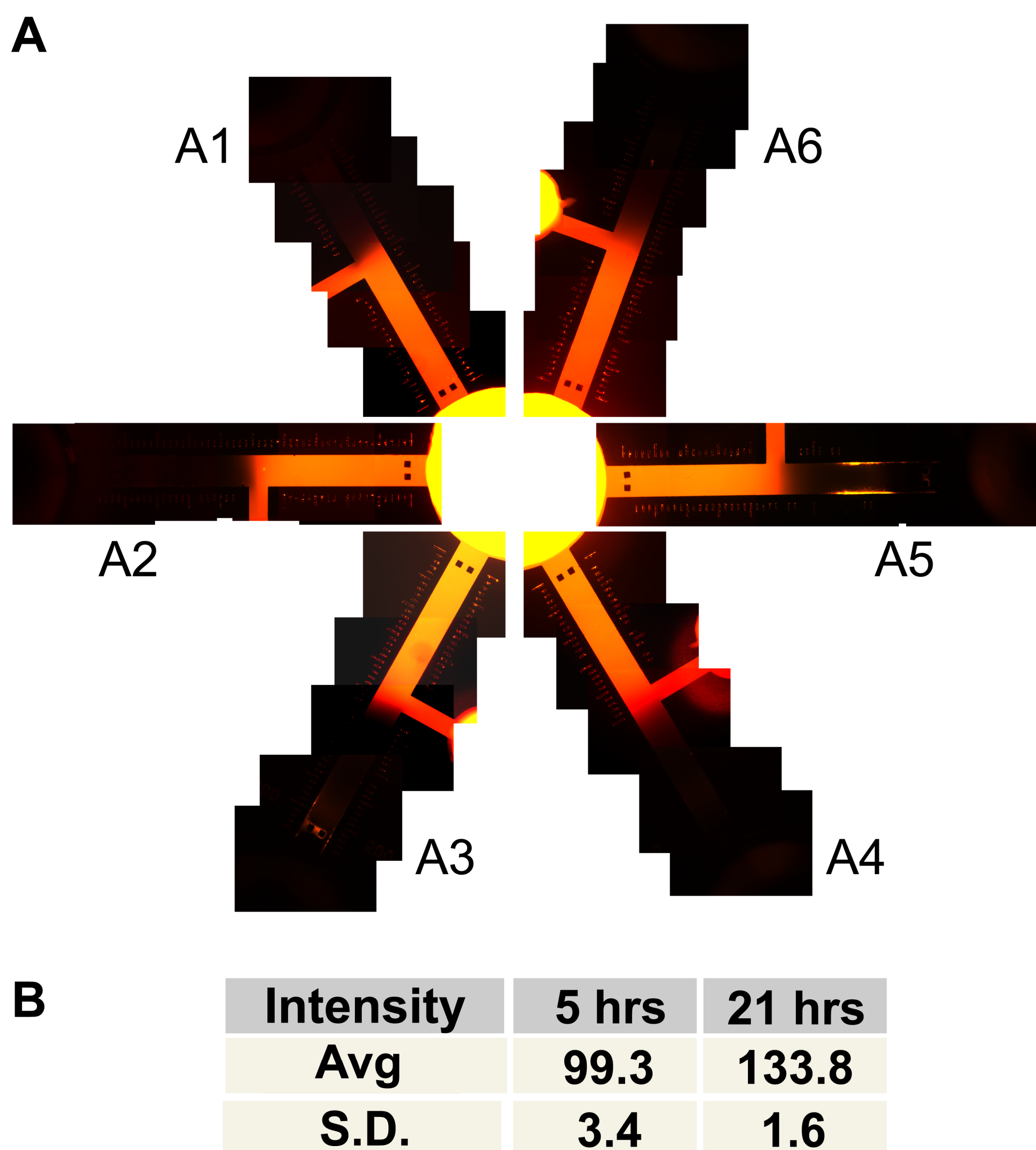
Supplemental Figures



Intensity	5 hrs	21 hrs
Avg	99.3	133.8
S.D.	3.4	1.6

Supplemental Figure 1. Diffusion profiles. **a:** Dextran conjugated with rhodamine B (10 kDa) was infused into the center chamber. The channels were filled with 0.5% agarose. The peripheral wells were filled with ultrapure water. Gradients formed in all channels over the observed 21 hrs. **b:** The average pixel intensity in all six channels was 99.3 after 5 hrs and 133.8 after 21 hrs. The modest standard deviations (3.4 and 1.6 respectively) demonstrate the suitability of the device for testing the cytotactic effects of a given factor on six different cell types. Values shown represent pixel intensity units.

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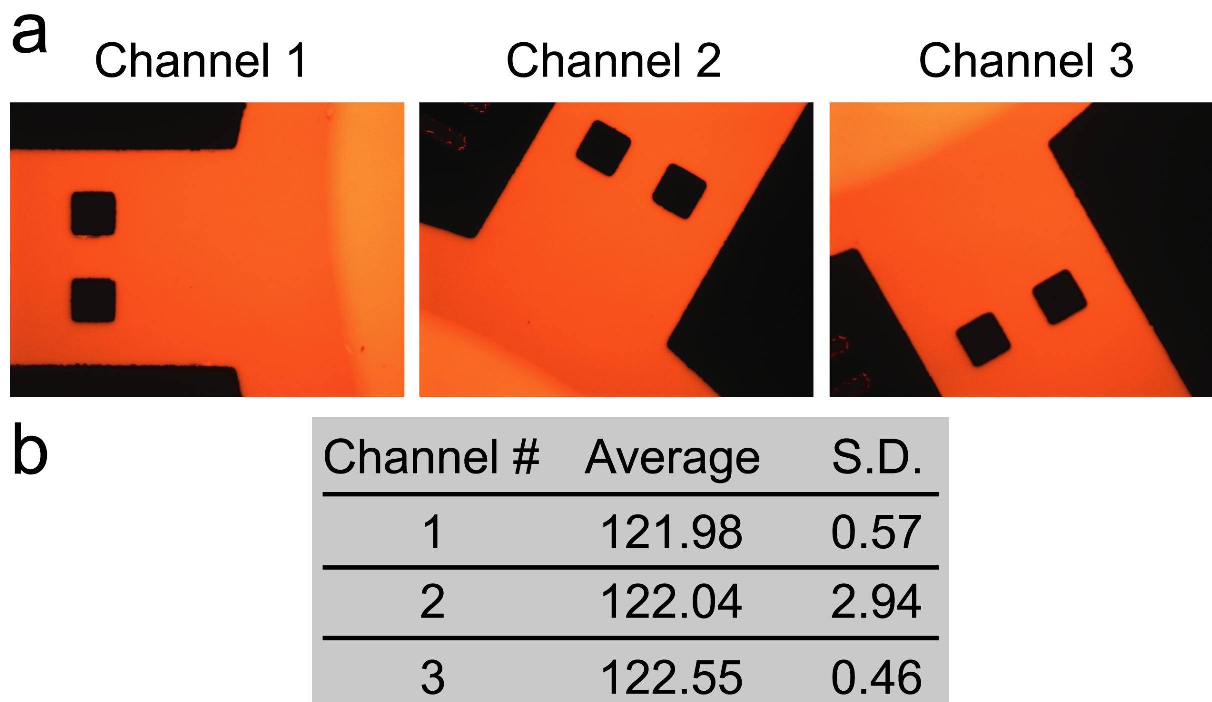

Channel #	Average	S.D.
1	121.98	0.57
2	122.04	2.94
3	122.55	0.46

Supplemental Figure 2. Consistency of fluorescent profiles between channels. **a:** Fluorescein conjugated with dextran was diffused through three separate channels and imaged after 21 hrs. **b:** The average pixel intensity of 15 selected locations in each channel and standard deviation are shown. No significant difference was found between the average pixel intensity in each channel, demonstrating the consistency in fluorescent profiles from channel to channel.

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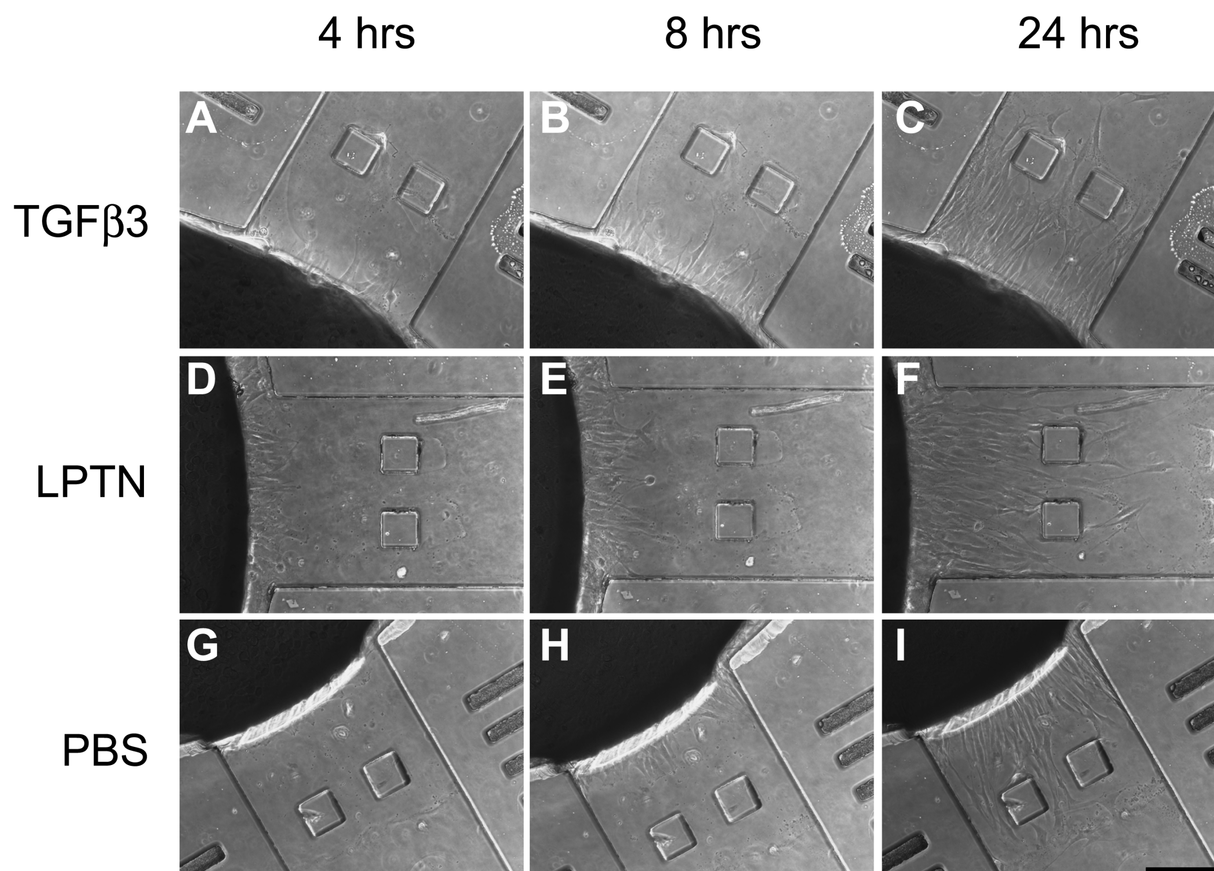

Supplemental Figure 3. Representative time-lapse images of induced cell migration. Cell migration was time-lapse imaged at 4 hrs (**a, d, g**), 8 hrs (**b, e, h**) and 24 hrs (**c, f, i**) after exposure to gradients of cytotatic cues. TGFβ3 (**a-c**), Lymphotactin (LPTN; **d-f**) and PBS (**g-i**; control) induced marked differences in the number and distances of cell migration after 24 hrs following infusion.

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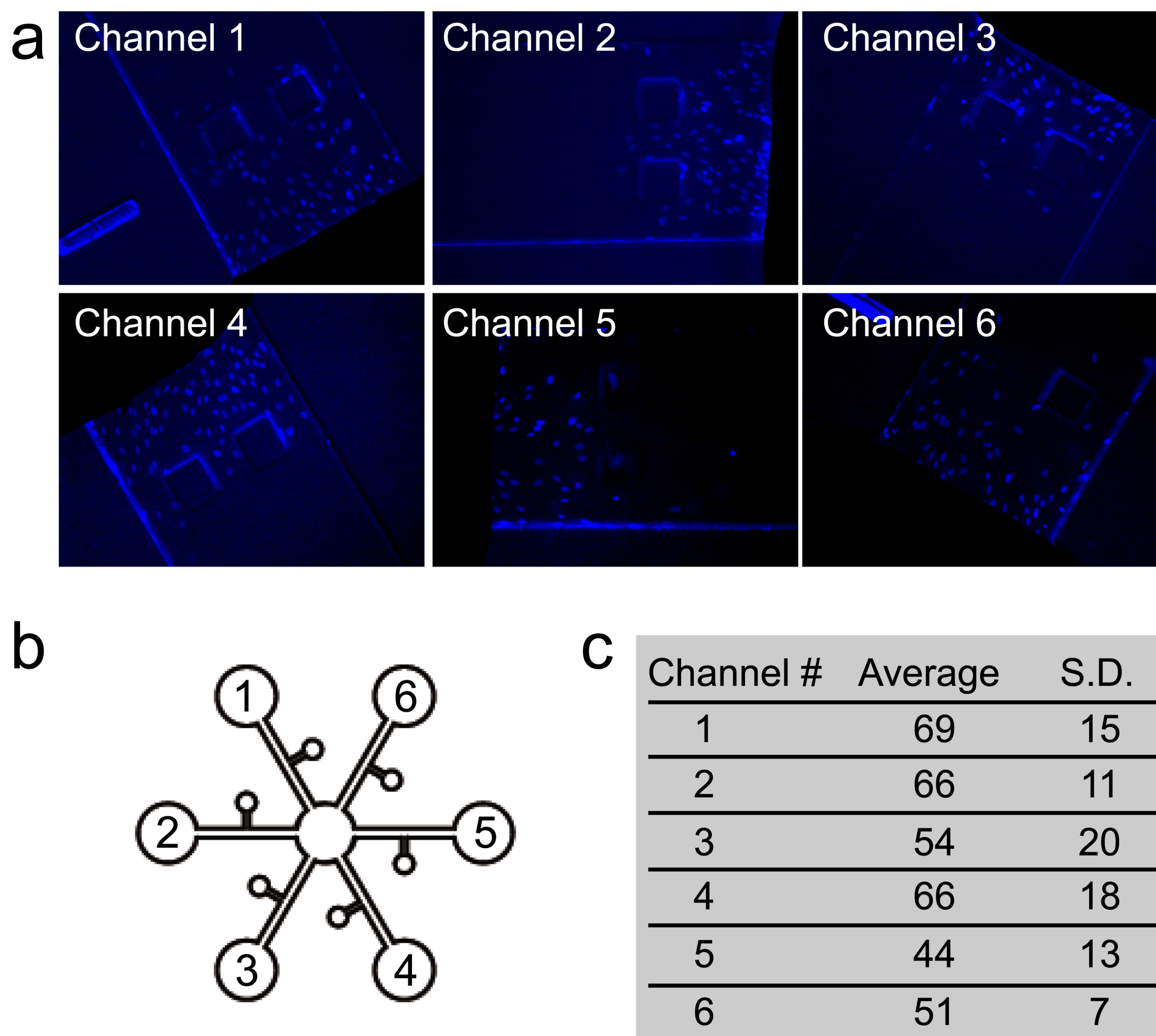

Channel #	Average	S.D.
1	69	15
2	66	11
3	54	20
4	66	18
5	44	13
6	51	7

Supplemental Figure 4. MSCs are not biased to migrate into a particular channel. The outlets of all 6 channels were filled with PBS and MSCs were allowed to randomly migrate for 24 hrs. **a:** DAPI stained MSCs that migrated into each channel of the device after 24 hrs. **b:** Schematic of the channel location corresponding to each channel number. **c:** The average number of cells per channel (n=3) and standard deviation were calculated. No significant difference was found among the averages in each channel, indicating that MSCs are not biased to migrate into any particular channel on the device.

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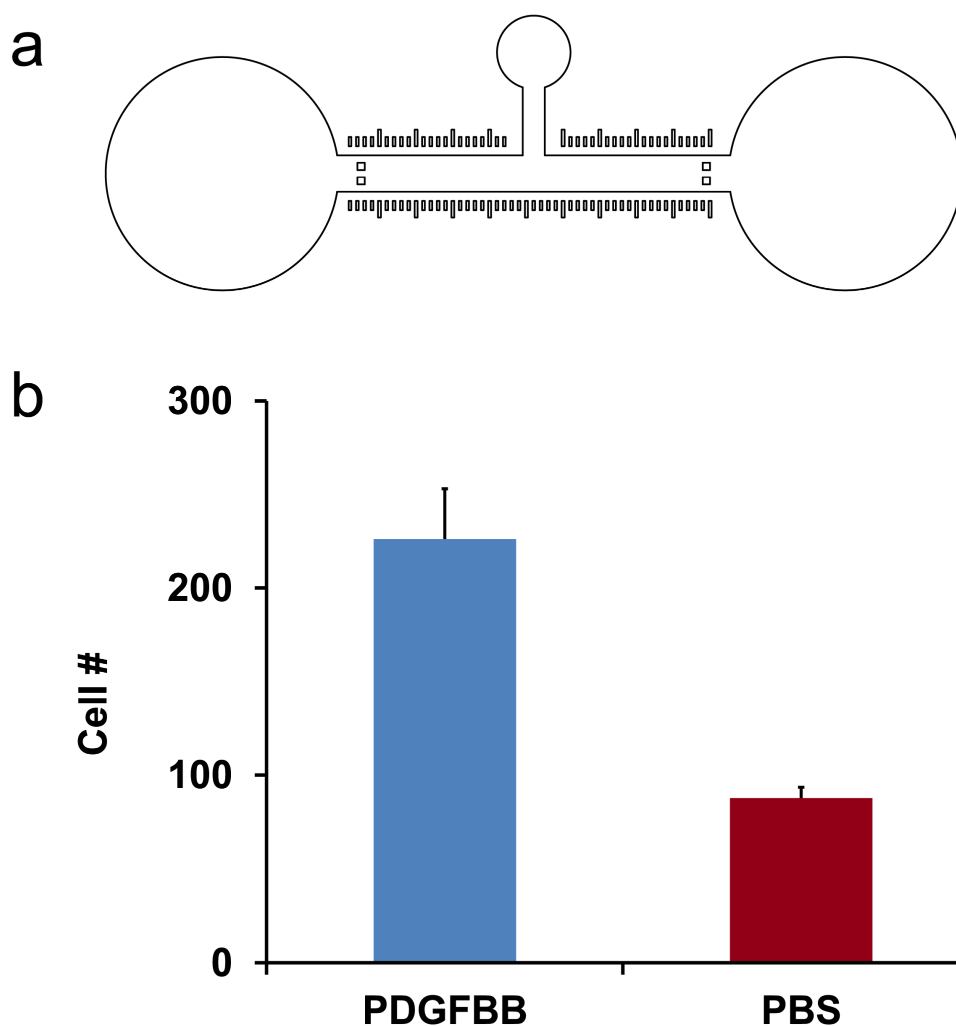

Supplemental Figure 5. PDGFbb effects on cell migration in a non-competition setting. **a:** A separate under-agarose migration device was fabricated with a single channel consisting of two chambers, one for cell seeding and the other for cytokine loading with the same configuration as a single unit of the 6-channel device. **b:** Significantly more MSCs migrated in response to PDGFbb (226 cells) compared to the control (88 cells) ($p<0.05$). Differences in cell migration in response to PDGFbb in a competition setting (122 cells in Group 1) compared to a non-competition setting (226 cells) indicate that the other factors in the 6-channel setting play interactive roles for cell migration.

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Sup. Movie 1. Cell migration is exemplified in response to IGF1. Cells seeded in the left chamber of the schematic below continuously migrate over the observed 12 hrs towards an increasing concentration of IGF1 (200 ng) that was infused in the right chamber. The ability to observe time-lapse images of cell migration is a distinct advantage of this device over conventional cell migration systems that only allow for the collection of endpoint data. Note that each cell was marked with a black dot to enhance visualization.